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A Non-social and Isolate Rearing Condition Induces an Irreversible Shift toward Continued Fights in the Male Fighting Fish (*Betta splendens*)

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ABSTRACT—Effects of rearing conditions were examined in the development of agonistic behaviors in the male fighting fish. In group-I (*highly social*), fish were communally reared. In group-II (*highly social and isolate*), fish were individually housed and exposed to the group-I fish through transparent walls until the sexual maturity (from 6 to 12 weeks post-hatch). In group-III (*social and isolate*), individually housed fish were similarly exposed to other fish within the group. In group-IV (*non-social and isolate*), individually housed fish were further visually isolated. Agonistic behaviors were compared among males of the groups-II, -III, and -IV in their fights against the group-I male. The group-IV males showed significantly higher rate of wins than the groups-II and -III males, without differences in the incidence of agonistic behaviors (butt-or-bite, chase, and gill-cover erect) before the termination of the mutual fights. Increased incidence of agonistic behaviors was found after the termination (particularly in the unilateral chase), suggesting that the group-IV males continued to fight even after the opponent male displayed a submission. The aggression was also enhanced in the group-II, when they were thereafter reared in a social isolation after the sexual maturation; a critical period was thus not found. The enhanced aggression was not reversed in the group-IV, when they were thereafter exposed to social stimuli; shift to the continued fights was irreversible. Possible fitness gain of the enhanced aggression was discussed in terms of the adjustability to altered biological resources.

Key words: Siamese fighting fish, agonistic behavior, social deprivation, adaptive socialization, fitness

INTRODUCTION

Animals must fight against their conspecifics to compete for biological resources such as foods, mates and habitat. A variety of species (ranging from aphids, warblers, to ground squirrels) thus have been known to perform extraordinarily high aggressive behaviors to defend their territory (Alcock, 2001). However, too excessive aggression will turn out to be maladaptive, since a considerable cost could outweigh the expected benefit; e.g., time and energetic cost consumed for aggressive behaviors, a risk of physical damages due to the fights, a risk of increased probability of predation, and lost opportunity for benefits that the animal could have gained if the efforts had been re-directed to foraging or mating behaviors. The cost grows larger as the animals continue to fight longer, so that they must stop the fight at a pivotal point where the expected benefit would balance the

accompanying cost of the fight. If the animals stopped too early, they would not gain the benefits at all and minimize the cost; on the other hand, if the animals continued the fight until too late, the accompanying cost would override the benefit even if they won; the animals should thus know the appropriate time to stop fighting. If the adaptationist hypothesis holds true, the key issue should be to understand how animals could adjust the time at the most optimized point in response to the environmental demands.

Actually, several studies have shown that an early deprivation of social stimuli leads to “maladaptive” development of excessive aggression in a variety of animals ranging from rhesus monkeys (Harlow and Harlow, 1962), blue gouramies (Tooker and Miller, 1980), to crickets (Nagao and Yamamoto, 1999). In the classical study, Harlow and Harlow showed that the rhesus monkeys reared in an extraordinary social deprivation (no contacts with mothers, conspecific juveniles, or other animals such as humans or dogs) failed to develop truly normal sexual, play and aggressive behaviors. Instead, the non-social isolate monkeys redirected their aggression toward themselves, so that a severe self-punish-

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ment occurred in response to appearance of an intruder. These effects were irreversible, and the monkeys could not be normalized even when they were afterwards treated in social environments. Similarly, in crickets, deprivation of physical contacts has been found to cause an excessive aggression in terms of increased rate of attacks, and also of misdirected attacks to females, often leading to the death of opponents. Could the changes have any fitness gains, or simply represent a maladaptive side effect of an adaptive trait?

The present study aimed at examining how environmental factors (particularly social factors during the early development) could change agonistic behaviors in the Siamese fighting fish (*Betta splendens*). The *Betta* is an anabantid native to Thailand, Cambodia, Malaysia and Myanmar. The males are known to have a characteristic repertoire of agonistic behaviors such as gill-cover erection (Simpson, 1968). The male fights are serious, and often involve physical damages to the opponent fish; on the other hand, females are smaller and duller in color, and do not fight so aggressively (Jaroensutasinee and Jaroensutasinee, 2001), suggesting a functional link between the aggression and the reproduction. Actually, males make and defend a bubble nest on the water surface, and solely provide parental care for developing eggs and fry. The nest-holding males become more aggressive (Jaroensutasinee and Jaroensutasinee, 2001; 2003), and the females choose mates according to the male-male competition. The male Siamese fighting fish is thus supposed to serve an ideal material for studying the adaptive aspects of aggressive behaviors. In this study, we have examined if an early social deprivation could change the fighting behaviors in adults, and if the changes could be reversed by social stimuli applied later in life.

MATERIALS AND METHODS

Subject fish and the rearing conditions

Experiments were conducted according to the guidelines of the Committee for Animal Experimentation of Nagoya University. Fry (~300) were hatched from a batch of fertilized eggs obtained from a pair of male and female purchased from a local supplier (Remix Co., Nagoya); therefore, all the subjects used in this study were genetically related at 0.25. The fry were isolated from the breeding male at 3 day after hatching, *i.e.*, when they started to swim. Thereafter, the fry were communally housed in tanks until 6 weeks post-hatch. The fry were fed with live brine shrimp, and subsequently with frozen red mosquito larvae. At 6 weeks, the standard length was measured, and a total of 110 large juveniles were selected for further experiments. The juveniles (the sex yet unidentifiable) were then allocated into the following 4 groups with different rearing conditions. The allocation was made randomly with their body length almost matched among the groups (Fig. 1A).

In group-I (*highly social group*), 5 fish were communally housed in a transparent plastic tank (12.5 cm × 20 cm, level of water was set at 6–9 cm). Ten sets of the group-I fish were prepared (50 fish in total). In group-II (*highly social and isolate group*), individual fish was housed in the same tank that was placed just beside the group-I tank; in this condition, the fish could see the population of group-I fish through the transparent walls. Twenty sets of the group-II fish were prepared (20 fish in total). In group-III (*social and isolate*

group), individual fish was similarly housed in the transparent tank and placed together with other tanks of the group-III; in this condition, the fish could see each other. Twenty sets of the group-III fish were prepared (20 fish in total). In group-IV (*non-social and isolate group*), individual fish was similarly housed in the transparent tank, which was visually isolated by surrounding opaque walls. Twenty sets of the group-IV fish were prepared (20 fish in total). Note that the other conditions were kept identical among the groups-II, -III and -IV. All of these tanks were perfused by a circulating flow of water supplied from a common reservoir tank (*ca.* 20 liters in volume), so that the temperature (thermo-controlled at 28±1°C) and chemical environments were basically identical. All the tanks were illuminated under a 12L:12D photoperiod. Fish were fed 6 times per week (*i.e.*, daily except Sundays) with frozen mosquito larvae; the food supply was gradually increased along the development from 0.1 g/day (at 6 weeks) to 0.5 g/day (at 12 weeks) per fish.

The juveniles were thus reared in these conditions until 12 weeks post-hatch, at which stage the sex became evident by their external appearances. We thus obtained 18 males and 32 females in the group-I, 13 males and 7 females in the group-II, 8 males and 12 females in the group-III, 9 males and 11 females in the group-IV, respectively. Body length (standard length, SL) and anal fin length were measured and compared among the males of the groups-II, -III, and -IV. The SL was compared in order to check the effects of rearing conditions on the growth, and the anal fin was compared to check the sexual maturation. Actually, different rearing conditions caused no significant differences in both measures when examined by one-way ANOVA (Fig. 1C and D); $F_{cal}(2,27)=1.81$, $p=0.18$ for the SL; $F_{cal}(2,27)=1.87$, $p=0.17$ for the anal fin length. Sexually matured females were reared together with the subject males, but not used in this study.

Tests of agonistic behaviors in the fight sessions

Experimental tank (20 cm × 30 cm, 15–16 cm deep) was initially partitioned into 2 chambers by a removable opaque wall. Aged tap water (thermo-controlled at 28±1°C) filled the tank, which was renewed at every fight session. The tank was surrounded by white wall, so that visual interference was minimized. A video camera was positioned in front of the tank, and the behaviors were recorded using a digital video recorder (DCR-TRV20, Sony Co.) for later analyses. Matched pair of the subject males were introduced to each of the two chambers and allowed to acclimatize for 30 min. Video record started, and 1 min later, the partition wall between the chambers was removed for the males to start fighting. Actually, fight started as soon as the wall was removed in most of the sessions observed in this study. In few cases at the tests 2-1 and 2-2 (see below), however, the group A fish did not fight from the very beginning. In such cases, we recorded the behaviors for 20 min and discarded the observations. The video record continued for 20 min after the beginning of the fight. When the distinction between a winner and a loser came out evident (*i.e.*, the termination of mutual fights) in 20 min, observation was completed at the point of 20 min. If otherwise, and the mutual fights continued further, the video record was stopped at the 20 min, and we observed the behaviors for a maximum of 2 hrs without making quantitative records. In case the mutual fights continued even at the 2 hrs, observations was completed and the session was noted as a draw. In each session, we recorded which male won, and also accomplished off-line quantitative analyses of the agonistic behaviors (frequency and duration) based on the recorded video data.

The following 3 types of agonistic behaviors were identified and counted in this study; butt-or-bite, chase, and gill-cover erect (Fig. 1B). Butt-or-bite: a fish rapidly approaches to another fish, then strongly butts or bites the opponent with its mouth. The mouth may nor may not be open at the time of physical contact. Butt-or-bite sometimes results in physical damages of the opponent's fins and scales. Chase: a fish rapidly chases a fleeing opponent. This uni-

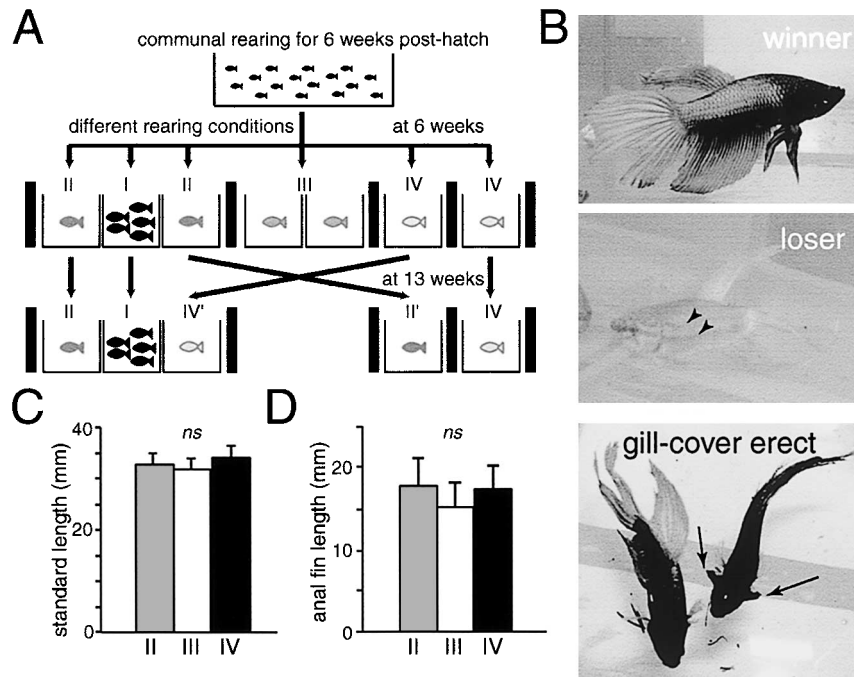


Fig. 1. Siamese fighting fish (*Betta splendens*) were reared in 4 different conditions during the period of sexual maturation (6–24 weeks post-hatch), and the reared males were tested for their agonistic behaviors; test 1 at 12–13 weeks, test 2-1 at 18–19 weeks, and test 2-2 at 23–24 weeks, respectively. **A:** Schematic drawing of the 4 rearing conditions. Group-I: 5 fish were communally housed in a transparent tank (*highly social group*). Group-II: individual fish was separated in a tank, which was placed beside a group-I tank (*highly social and isolate group*). Group-III: individual fish was separated in a tank, which was placed by another group C tank (*social and isolate group*). Group-IV: individual fish was separated in a tank, which was visually isolated by opaque walls (*non-social and isolate group*). Fry were communally reared for 6 weeks post-hatch, and the juveniles (the sex yet unidentifiable) were randomly allocated into these groups with the matched body sizes. After the test 1 (at 13 weeks), some of the group II and IV fish was replaced with each other, and named as II' and IV' fish, respectively. **B:** Photographs of sexually matured males (13–18 weeks old) in 3 behavioral states. Top: a winner male displaying the darkened body color and widely extending the pectoral, anal, dorsal and tail fins. Middle: a loser male with a reduced coloration of the body; note also that the horizontal stripes on the trunk became apparent (arrowheads). Bottom: a male on the right is threatening another male on the left by erecting its gill-covers on both sides (arrows). **C, D:** Different rearing conditions resulted in no significant differences in the standard length (**C**) and the anal fin length (**D**). Columns and bars indicate means and s.e.m., respectively; ns means no statistical significance detected by one-way ANOVA at $p < 0.05$.

lateral attack is not accompanied by any physical contacts. Gill-cover erect: a fish widely erects the gill-covers on both sides, and displays the posture toward the opponent. No physical contacts accompany. In some cases, both of the matched males show the gill-cover erect toward each other for several min; we therefore measured the cumulative duration (sec/min) of this behavior, rather than the frequency.

The subject males were tested in their fights at 12–13 weeks post-hatch (test 1). Of the fish available, we selected 8 males from each of the 3 experimental groups-II, -III, and -IV as subjects, and 16 males from the group-I as opponents, respectively. The SL of these fish did not significantly differ among the experimental groups; $F_{ca}(2,21)=1.78$, $p=0.19$. In each fight session, a male of an experimental group was randomly matched with a group-I male of a similar SL; a maximum SL difference of 3.6 mm was allowed between the matched males. We thus obtained data from 11 sessions (II vs. I), 10 sessions (III vs. I), and 11 sessions (IV vs. I), respectively. In these 32 sessions, 3 (2, or 3) males in the group-II (-III, or -IV) were examined twice, and all of the 16 males in the group-I were recruited twice.

The males were further tested at 18–19 weeks (test 2-1) and 23–24 weeks (test 2-2), after half of the group-II and -IV males were exchanged at 13 weeks post-hatch. Seven out of the 13 males of group-II were placed in the group-IV condition (*non-social and isolate*) and named as group-II'; the remaining 6 males remained as group-II. Similarly, 5 out of the 9 males of group-IV were placed in

the group-II condition (*highly social and isolate*) and named as group-IV'; the remaining 4 males remained as group-IV. We thus made 4 experimental groups (II, II', IV, and IV') and tested them in their fighting behaviors with 16 or 15 males of the group-I as opponent; one of the group-I males died between the tests 2-1 and 2-2. At the test 2-1, we obtained data from 11 sessions (II vs. I), 10 sessions (II' vs. I), 10 sessions (IV vs. I), and 10 sessions (IV' vs. I). At the test 2-2, we obtained data from 12 sessions (II vs. I), 10 sessions (II' vs. I), 9 sessions (IV vs. I), and 8 sessions (IV' vs. I). Note that the subject males were examined at least twice, and the opponent group A males were repeatedly recruited ~3 times.

RESULTS

Comparisons among the groups -II, -III, and -IV at test 1: effects of the early social isolation on the agonistic behaviors

In the non-social / isolate group (group-IV), the males won in all of the fight sessions (Table 1). On the other hand, in the social groups (group-II and -III), around the half of the males won; chi-square test among the groups-II, -III, and -IV revealed a significant difference in the win rate (*i.e.*, the number of winners and losers in fight sessions; the draw

cases were not included). No significant differences occurred among the groups in the time of termination (*i.e.*, when distinction between the winner and the loser became evident; Fig. 1B), and also in the frequencies of butt-or-bite and in the duration of gill-cover erect before termination (Fig. 2A,C). It is therefore suggested, though it may sound a paradox, that the group-IV males won the fights without enhancement in the preceding agonistic behaviors. In other

words, incidence of agonistic behaviors does not give a good measure as to which one will finally win the fight. In the agonistic behaviors after the termination, on the other hand, the group-IV fish showed significantly higher incidence than the groups-II /III; data were merged because the males of these 2 groups showed similar rate of wins. Note the significantly higher frequencies of the butt-or-bite and of the chase after termination (Fig. 2A,B), which indicate that the

Table 1. Results of the fight sessions in the test 1. Males of the non-social and isolate group won in most of the fight sessions (group-IV). In each of these fights, a male of the group-II (-III or -IV) was paired with a group-I male in an experimental tank. No significant difference was found in the time of termination; median values were shown together with the range (min-max), and *ns* means $p>0.05$.

groups	Test 1 at 12–13 weeks			
	winner	loser	draw	time of termination (sec)
II	4	5	2	446.0 (16-4200, $n=9$)
III	6	4	0	285.5 (158-4200, $n=10$)
IV	10	0	1	197.0 (55-6600, $n=10$)
Statistics	0.05> $p>0.01$ ($\chi^2_{cal} = 7.34$) χ^2 -test			ns ($H_{cal} = 1.54$) Kruskal-Wallis test

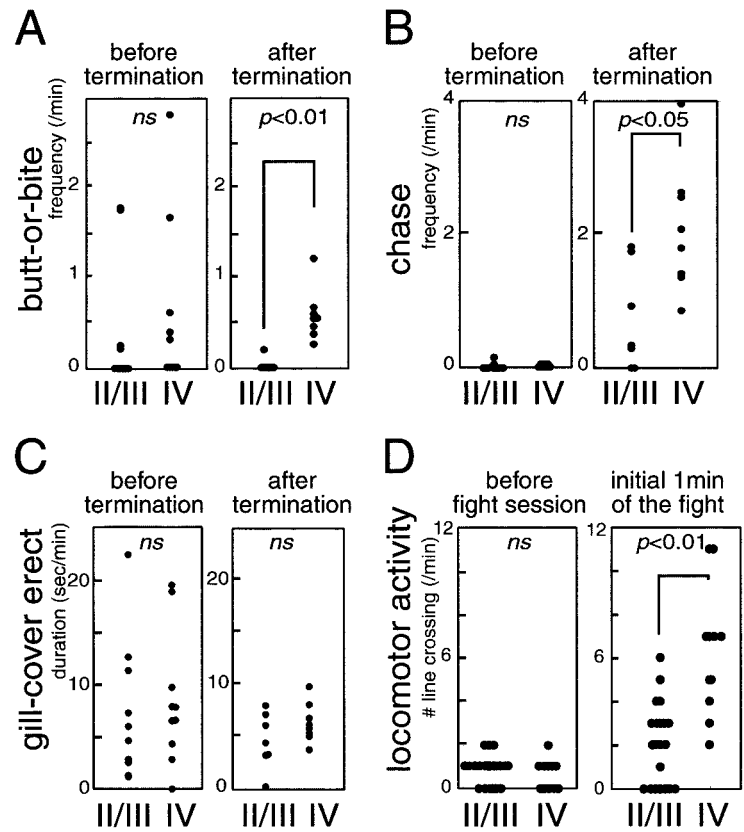


Fig. 2. Quantitative comparisons of the fights observed in the test 1. When compared with the social and isolate males (groups-II /III; data from these two groups were merged), the non-social and isolate males (group-IV) showed significantly higher scores in the frequency of the butt-or-bite (A) and the chase (B), but not in the duration of gill-cover erect (C), in the episodes after the termination of mutual fights. Spontaneous locomotor activities measured by the number of center-line crossing per min (D) were also compared between the period of 1 min just before the beginning of fight sessions, and the period of 1 min just after the beginning of the fight. Note the group-IV fish showed significantly enhanced locomotor activity selectively in the initial 1 min of the fight session. One symbol denotes data obtained from one fighting session in these and the following figures. Mann-Whitney's U-test was used to compare data between II /III and IV at $p<0.05$; *ns* means no significant differences.

group-IV males committed unilateral attacks even after the loser had already displayed a submission. It is also to be noted that the group-IV fish showed significantly higher spontaneous locomotor activities than the groups-II /III; the enhanced locomotion was observed in the initial 1 min of the fight session, and not in the period of 1 min just before the partition wall was removed (*i.e.*, the start of the fight session) (Fig. 2D). We could therefore assume that the enhanced win rate could be ascribed to some aspects of non-agonistic behaviors such as the hyperactivity. However, this proved not to be a plausible explanation, because the enhanced locomotion was not reproduced in the tests 2-1 and 2-2 (see below; Fig. 4).

Comparisons between the groups-II and -II' at test 2-1 and 2-2: effects of the late isolation on the agonistic behaviors

Is a social isolation after the period of sexual maturation also effective in enhancing the win rate? To answer this question, some of the group-II males were placed in the group-IV condition, reared for 5 to 10 weeks, and tested again (Table 2). The allocation to the groups-II and -II' was performed in reference to the results at the test 1, so that the proportions of the winners were almost identical between these two groups. At the test 2-1, the group-II' males (non-social / isolation rearing from 13 to 18 weeks post-hatch) actually showed an enhanced win rate than the group-II (social / isolation rearing up to 18 weeks post-hatch), although the difference was suggestive ($p=0.068$) but not significant by the Fisher's exact probability test. At the test 2-2, on the other hand, even the group-II males won in most of the fight sessions, without showing a difference between the groups-II and -II'. It is also noted that the time

of termination was much shorter in the test 2-2 than the test 2-1; $U_{cal}(20, 18) = 81.0$, $p=0.0062$ (Mann-Whitney's U-test). Most probably, the group-I males changed their behaviors and turned to lose much easier and earlier at test 2-2 performed at 23–24 weeks post-hatch; no conclusions could thus be gained from the data obtained at the test 2-2 as to the behaviors of the group-II'. Similarly to the test 1, no significant differences were found in the incidence of agonistic behaviors (butt-or-bite, chase, and gill-cover erect) before the termination (Fig. 3A). After the termination, on the other hand, the group-II' males showed a significantly longer duration of gill-cover erect than the group-II males, though no differences were found in the butt-or-bite and the chase. In conclusion, rearing in social isolation after the sexual maturation tended to enhance the win rate without significant effects on the incidence of agonistic behaviors, similarly to the effects found in the test 1.

Comparisons between the groups-IV and -IV' at tests 2-1 and 2-2: effects of the late socialization on the enhanced agonistic behaviors

Could the enhanced win rate be reversed if they were thereafter reared in a highly social environment? Or otherwise, could the enhancement remain irreversibly? To answer this question, some of the group-IV males were placed in the group-II condition, reared for 5 to 10 weeks, and tested again (Table 2). At the test 2-1, the group-IV' males (highly social and isolate rearing from 13 to 18 weeks post-hatch) remained to show an enhanced win rate similar to the group-IV (non-social and isolate rearing up to 18 weeks post-hatch) without a significant difference. Between the groups-IV' and -IV, no differences were found in the

Table 2. Results of the fight sessions in the tests 2-1 and 2-2.

groups	Test 2-1 at 18–19 weeks				Test 2-2 at 23–24 weeks			
	winner	loser	draw	time of the termination (sec)	winner	loser	draw	time of the termination (sec)
II	7	4	1	297.0 (32-432, n=11)	7	1	4	87.5 (24-943, n=8)
II'	9	0	2	826.0 (58-4800, n=9)	10	0	0	67.0 (19-455, n=10)
Statistics	ns ($p=0.068$) Fisher's exact probability test		–	ns ($U_{cal}=31.00$) Mann-Whitney's Utest	ns ($p=0.89$) Fisher's exact probability test		–	ns ($U_{cal}=35.00$) Mann-Whitney's Utest
groups	Test 2-1 at 18–19 weeks				Test 2-2 at 23–24 weeks			
	winner	loser	draw	Time of termination (sec)	winner	loser	draw	Time of termination (sec)
IV	10	0	0	178.0 (41-919, n=10)	9	0	0	44.0 (24-355, n=9)
IV'	9	0	1	257.0 (51-1177, n=9)	8	0	0	70.5 (20-280, n=8)
statistics	ns ($p=0.37$) Fisher's exact probability test		–	ns ($U_{cal}=43.00$) Mann-Whitney's Utest	ns ($p=1.00$) Fisher's exact probability test		–	ns ($U_{cal}=23.00$) Mann-Whitney's Utest

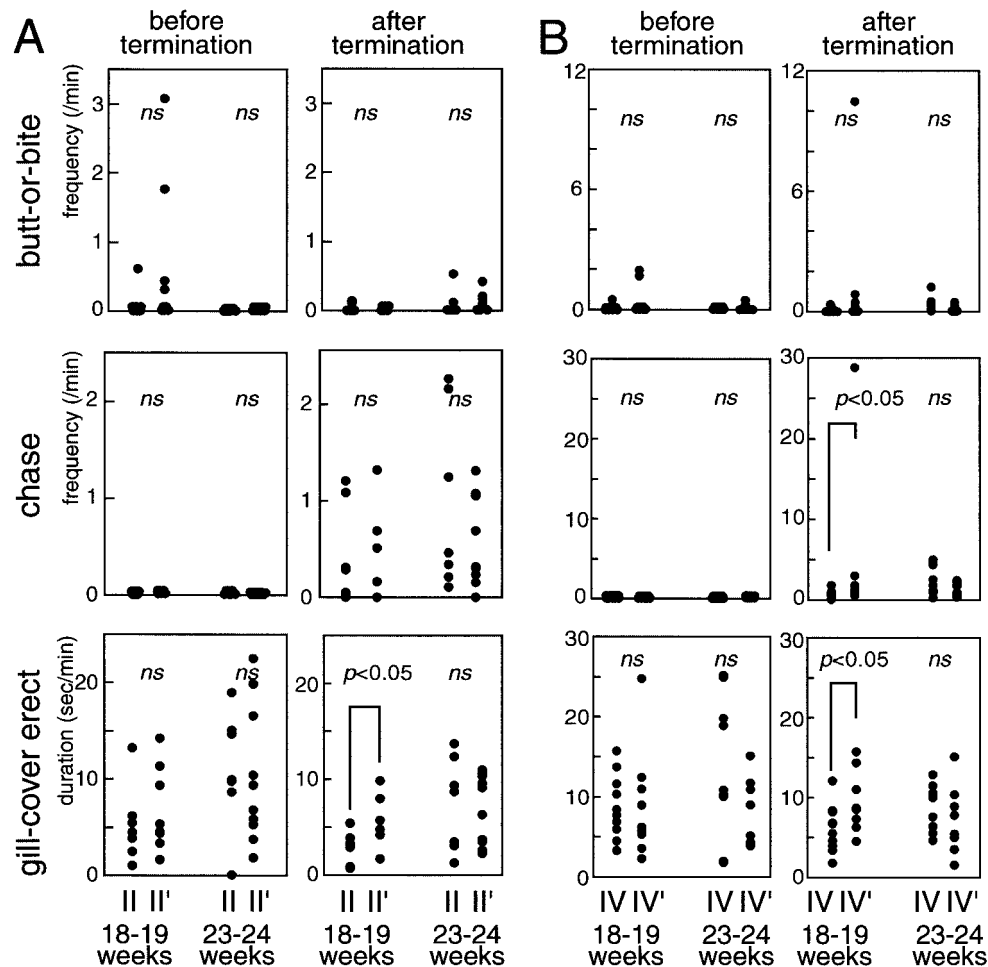


Fig. 3. Quantitative comparisons of the fights observed in test 2-1 and 2-2; no significant changes occurred after the exchange between the social and isolate condition (B) and the non-social condition (D) in their behaviors during the episodes before the termination of mutual fights. Comparisons were made between II vs. II' (A), and between IV vs. IV' (B). After the termination, an enhancement appeared in the gill-cover erect between II vs. II', and the chase and the gill-cover erect between IV vs. IV'. Mann-Whitney's U-test was used at $p < 0.05$.

time of termination, as well as in any of the agonistic behaviors before termination (Fig. 3B). After the termination, however, the group-IV' males showed significantly higher scores in the chase and the gill-cover erect. At the test 2-2, similarly, the time of termination was significantly shorter than the test 2-1; $U_{cal}(19, 17) = 69.0$, $p = 0.0034$ (Mann-Whitney's U-test). We thus do not gain a conclusion from data obtained at the test 2-2. In conclusion, we can conclude that a rearing in highly social condition does not reverse the enhanced win rate.

Social isolation and enhanced locomotion

In the test 1, as has been pointed out, the non-social and isolate group-IV males showed significantly higher locomotion (number of the center line crossing in the experimental tank) than the groups-II /III males during the initial 1 min of the fight session. Such an enhanced locomotion was not observed at tests 2-1 and 2-2 (Fig. 4), in which the group-IV males showed low numbers of line crossing similar to the other groups (-II, -II' and -IV'). We therefore conclude that

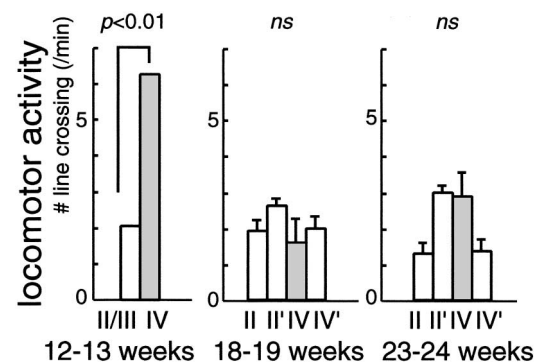


Fig. 4. Comparisons of the spontaneous locomotor activity (number of center line crossing) during the period of the initial 1 min of the fight session revealed an enhancement in the test 1, but not in the tests 2-1 and 2-2; columns and bars indicate the mean and the s.e.m., respectively. Filled columns indicate data obtained from the group-IV. One-way ANOVA (or t-test for the data at 12–13 weeks) was applied at $p < 0.05$.

the enhanced locomotion was a transient effect, and not causally linked to the enhanced win rate.

DISCUSSION

The present study suggests that an early social isolation (the group-IV), but not the physical separation that accompany (as in the groups-II and -III), enhances the win rate (Table 1) without changes in the preceding agonistic behaviors (Fig. 2). Increased incidence of agonistic behaviors after the termination of mutual fights suggest rather that the increased win rate was due to the inability of the group-IV males to stop fighting at an earlier point of time. In the groups-II and -III, on the other hand, the males had been exposed to the other individuals through transparent walls of the rearing tanks, so that they could have a chance to learn the appropriate point of time to stop fighting. This idea still remains one of possible explanations, and needs to be examined in a simple test where fights between the group-IV males are analyzed for the time of termination of mutual fights; a prediction is that they will continue to fight for a much longer period of time than the pairs matched among the groups-II/III males.

Alternative explanation is that, instead of assuming an enhancement, we could also assume that the social interactions suppressed the agonistic behaviors in the groups-II and -III. Halperin and Dunham (1994) have actually reported a waning of intense aggression in *Betta splendens*, when the males were continuously subjected to social stimulation, or "social overstimulation". In their study, adult males were surrounded by conspecifics for several weeks, and subsequently showed a reduced rate of the bite, but not the gill-cover erect; the phenomena distinct from those found in our present study. The reduced aggression found in the group-I at test 2-2 could be compatible with their findings, although our main finding in the group-IV males cannot be ascribed to the reduced aggression.

We also found that the enhanced win rate remained even after the group-IV males had been thereafter reared for 5 weeks in a social condition (Table 2). On the other hand, socially reared males of the group-II also developed an enhanced win rate after they had been reared for 5 weeks in a non-social isolate condition, again without accompanying increases in the incidence of agonistic behaviors before the termination of mutual fights (Fig. 3A). These results lead us to conclude that; (1) development of the enhanced aggression is irreversible, and (2) there is no specific period of life that is critical for the behavioral plasticity (or the critical period). Under natural environments, the amount of social interactions would give a good measure about the estimated amount of benefits available when the male won the fight; a lower interaction could be linked to a lower density of opponents in the field, thus enabling a larger territory and the accompanying resources for the winner. The males thus reared could continue fighting for a longer period of time, if the estimated high benefits still outweigh the

increased cost after the long fight. We should examine this possibility by specifically controlling the resources available, such as the food or the sexually matured females in the rearing and the experimental tanks.

Similar enhancements in the aggressive behaviors have been reported in a variety of fish. In a mouth-breeding cichlid fish (*Haplochromis burtoni*), Fernö (1977) reported an enhanced number of attacks after a short separation for 15 min to 12 days, though a longer isolation reversed the effects and the attack rate was even decreased afterwards, suggesting a complex of multiple processes with short- and long-term effects. In a more recent study on another cichlid fish (*Oreochromis niloticus*), Barki and Volpato (1998) report an effect of early social environments (*i.e.*, a lack of interactions with conspecifics with a normal fin display) markedly increased the onset latency of aggressive behaviors, without accompanying changes in the rate and sequences of the behavior patterns. These reports, together with the present results, suggest that the long-term effects of social environment could largely vary depending on the ecological and behavioral background of the animal under study. Definitely, further studies are required to reveal the possible fitness of the altered aggression.

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REFERENCES

- Alcock J (2001) Animal Behavior. Sinauer Associates, Sunderland, Massachusetts
- Barki A, Volpato GL (1998) Early social environment and the fighting behaviour of young *Oreochromis Niloticus* (pisces, cichlidae). Behaviour 135: 913–929
- Fernö A (1977) The effect of social isolation on the aggressive and sexual behaviour in a cichlid fish, *Haplochromis burtoni*. Behaviour 65: 43–61
- Halperin JRP, Dunham DM (1994) Social overstimulation reduces subsequent aggression in *Betta splendens*. Aggres Behav 20: 135–142
- Harlow F, Harlow MK (1962) Social deprivation in monkeys. Sci Am 207: 137–146
- Jaroensutasinee M, Jaroensutasinee K (2001) Sexual size dimorphism and male contest in wild Siamese fighting fish. J Fish Biol 59: 1614–1621
- Jaroensutasinee M, Jaroensutasinee K (2003) Type of intruder and reproductive phase influence male territorial defence in wild-caught Siamese fighting fish. Behav Proc 64: 23–29
- Nagao T, Yamamoto S (1999) Effects of social experience on aggressiveness and development of the sexual behavior in male crickets. Comp Biochem Physiol A 124 (suppl 1): S57
- Simpson MJA (1968) The display of the Siamese fighting fish, *Betta splendens*. Anim Behav Monog 1: 1–73
- Tooker CP, Miller RJ (1980) The ontogeny of agonistic behaviour in the blue gourami, *Trichigaster trichopterus* (Pisces, Anabantoidae). Anim Behav 28: 973–988

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